IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. 10/677,734	Customer No. 23379
Applicant: Gardner et al.	Confirmation No. 4912
Filed: Oct 01, 2003	Group Art Unit: 1656
Docket No. UTSD:1510-1	Examiner: Swope, Sheridan

Title: *Foreign PAS Ligands Regulate PAS Domain Function*

DECLARATION UNDER 37CFR1.132

I, Professor Kevin H. Gardner, declare and state as follows:

1. I am an Associate Professor of Biochemistry and Pharmacology at the University of Texas Southwestern Medical Center in Dallas, where I also serve as the Chairman of the Molecular Biophysics Graduate Program. The Board of Regents of the University of Texas System is the assignee of this patent application. I have authored numerous scientific papers in the field of NMR analyses of protein structure, function and regulation, and I am particularly knowledgeable in the field of PAS domain structure and function. I am a coinventor on this patent application, and have read and considered the Decision dated Jul 15, 2008.

2. In his declaration dated Jun 19, 2006 in this application, my colleague, Professor Stephen R. Sprang explained why it is his expert opinion that one skilled in the art at the time of the filing date of this application would not have expected HIF2a PAS to provide a core for sensory ligand binding. I agree with Professor Sprang's reasoning and conclusion, and I believe that those skilled and knowledgeable in the art would also agree with him.

3. To my mind there is an unsettling disconnect between the USPTO's statements regarding obviousness in this application to date, and what those skilled in the art in fact understood and expected. This declaration is intended to help the USPTO better understand, and hopefully better reflect the perspective of one skilled in the art at the time the invention was made.

4. The Introduction section of our patent application accurately summarizes the historical context:

> Some members of the PAS family are known to contain small molecules within their cores, allowing them to sense stimuli and regulate diverse biological processes. For example, heme binding by the PAS domains of FixL (Gong et al., 1998; Miyatake et al., 2000) and Dos (Delgado-Nixon et al., 2000) allows bacteria to sense oxygen levels; blue light photoreception in plant phototropins is achieved through a flavin molecule associated with their LOV domains (a PAS domain

subclass) (Crosson et al., 2003); and binding of exogenous organic compounds by the C-terminal PAS domain of the aryl hydrocarbon receptor (AhR) displaces a chaperone protein, induces a conformational change and activates the transcription of xenobiotic metabolizing enzymes (Schmidt & Bradfield, 1996). In all these examples, the cofactor is reportedly required for proper folding and functioning of the PAS domain within the context of the holo-protein.

However, for most PAS domains there is no evidence for such a cofactor. In fact, structurally characterized PAS domains without bound cofactors (Amezcua et al., 2002; Erbel et al., 2003; Morais Cabral et al., 1998) show tightly packed cores with no pre-formed cavities that would suggest a cofactor or ligand binding site.

Specification, p.1, line 22 – p.2, line 5.

5. At the time my laboratory published Amezcua (Structure 10, 1349-61,2002) it was widely and generally believed that there were two distinct and mutually-exclusive types of PAS domains: those with an endogenous core cofactor and those without. Those with cofactors were found to require the cofactor for folding into the PAS structure and for maintaining structural stability. The cofactors were structurally confined in an open pocket within the hydrophobic core of the domain that was discernable by NMR or crystallographic analysis. In contrast, those PAS domains without endogenous cofactors did not present such a core pocket. In other words, some PAS domains required cofactor to fold into the PAS structure, and others did not:

> PAS domains, as with many other classes of signal transduction modules, are protein/protein interaction domains that are used for intra- and intermolecular associations of macromolecules. To regulate such interactions in response to environmental factors, many PAS domains have integrated organic cofactors within their hydrophobic cores as sensors. Changes in the conformation or occupancy of these cofactors signal changes in stimuli that cannot be adequately sensed by moieties composed solely of the twenty natural amino acids. For example, PAS domains have been demonstrated to use heme to sense O_2 in several bacterial enzymes [3], FMN to sense blue light in plant phototropins [4], and 4-hydroxycinnamic acid to sense blue light in photoactive yellow protein [5]. It is intriguing to note that, from a structural standpoint, these diverse ligands are tolerated within the cores of different PAS domains, all of which retain the same mixed α/β fold diagrammed in Figure 1A. Folding into this structure is not dependent on ligand binding in all cases, though, as demonstrated by the well packed, ligand-free hydrophobic core observed in the crystal structure of the human *ether-a-go-go*-related gene (HERG) PAS domain [6].
> Amezcua 2002 (supra) at p.1349, para bridging col.1-2.

6. That the two types of PAS domains were mutually exclusive was consistent with the evidence at the time. For example, no one had obtained structures of both members of cognate pairs of the same PAS domain with and without ligand, suggesting that the cofactored PAS domains were unstable and did not adopt unique, well-defined conformations in their "apo" (cofactor-free) forms.

7. This expectation was also consistent with experience with the AhR PAS2 domain wherein the "apo" form was found only in association with a stabilizing chaperone, heat shock protein 90

(Hsp90). Without the stability of the chaperone complex, the AhR PAS domain is structurally unstable and unable to bind ligand:

> In the cytosol, the unliganded AhR is found in a complex with a dimer of the heat shock protein hsp90 and a 43-kDa protein, p43 (248, 249). hsp90, which is a molecular chaperone, is apparently required for maintaining AhR in a nonactivated, ligand-binding conformation (179) ... The PAS2 but not the PAS1 domain (residues 230 to 421) is required for ligand or hsp90 binding to AhR (Fig. 17) (39). The chaperone role of hsp90 appears to prevent premature dimerization of the receptor to DNA-binding partners and assists in ligand binding to AHR by ensuring proper folding of the ligand-binding domain (39, 193).
> Taylor et al., 1999, Microbiol. Mol. Biol. Reviews, 63, 479-506,500, col.1.

8. Furthermore, NMR and crystallographic data distinguished the two types of PAS domains: the cofactored class (such as PYP and AhR) showed a hydrophobic core pocket (containing the cofactor), and the non-cofactored class (such as HERG and HIF2a PAS B) showed tightly packed cores. For example, PYP has a covalently bound core chromophore ligand (trans-p-hydroxycinnamic acid, and the crystal structure shows the chromophore within a hydrophobic core pocket; Cusanovich et al (2002, Biochemistry 42, 4759; Fig. 3).

9. In the course of determining the solution structure of PASK, my laboratory determined that several regions of hPASK PAS A are unusually flexible. From our finding of "unusual flexibility of hPASK PAS A near the ligand binding sites of the FixL and Phy3 domains [13,14,19], we hypothesized that hPASK PAS A might bind small organic compounds." Amezcua 2002 (supra, 1352, col.1). In contrast, the sequence of HIF2a PAS B (Tian et al., Genes Dev. 1997 Jan 1;11(1):72-82) did not indicate the presence of any such extended loops, dynamic regions or unusual flexibility as we observed with PASK PAS-A. Finally, it was known that the oxygen-dependent transcriptional regulation conferred by HIF2a was encoded in protein regions outside the PAS-B domain (O'Rourke et al., J. Biol. Chem. 1999, 274: 2060-2071), further lowering expectations that the PAS domains themselves bound any oxygen-sensitive ligand or cofactor.

10. Based on the foregoing and the general knowledge in the field at the time, it is my opinion that one skilled in the art at the time of the filing date would not have expected HIF2a PAS to provide a core for sensory ligand binding.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: Sep 15, 2008

Prof. Kevin H. Gardner